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(Problem 2)

STUDY PLAN

POSSIBLE ANTAGONISM OF THE BLUESTAIN FUNGUS, *CERATOCYSTIS MINOR*, BY OTHER MICROORGANISMS ASSOCIATED WITH THE SOUTHERN PINE BEETLE

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By

J. Robert Bridges and Thelma J. Perry

INTRODUCTION

The symbiotic association between the southern pine beetle, *Dendroctonus frontalis* Zimm. (SPB), and the bluestain fungus, *Ceratocystis minor*, has long been known. The fungus depends on the insect for dissemination and introduction into susceptible trees (Nelson 1934). Whether the beetle benefits from the association has not been unequivocally demonstrated, but bluestain infection causes a reduction in moisture content of the tree and may create conditions that favor brood development (Nelson 1934, Nelson and Beal 1929, Bramble and Holst 1940, Leach *et al.* 1934). However, where the inner bark has been extensively colonized and stained by *C. minor*, larvae make atypically long galleries and develop poorly. The life cycle is extended, and complete development may never occur (Barras 1970, Franklin 1970).

Ceratocystis minor is apparently inhibited in active beetle galleries. Isolations made from early larval mines yield several yeasts and bacteria (Barras and Marler 1974, Marler and Barras 1978, Moore 1972a & b, Callahan and Shifrine 1960, Shifrine and Phaff 1956), but *C. minor* is only rarely found (Oberle 1966, Barras 1970). But, after larvae have entered the outer bark to pupate, *C. minor* can be consistently isolated from the abandoned galleries. We speculate that microorganisms growing in SPB galleries inhibit *C. minor* growth by competing for nutrients or space or by producing antibiotic compounds. Interactions among microorganisms involving competition and antagonism are well documented (Shigo 1965, 1967; Blanchette and Shaw 1978; Barras 1969), although interactions among microorganisms associated with the SPB have not been studied.

The purpose of this study is to investigate whether *C. minor* growth or sporulation is inhibited by the other microorganisms associated with SPB. Interactions among organisms will be studied on agar plates as well as in the tree.

MATERIALS AND METHODS

Possible antagonists to *C. minor* will be isolated from the SPB, its frass, and its galleries. Most microorganisms will be those isolated in another study.[1/]

To study interactions on agar plates, *C. minor* and the candidate microorganism will be cultured 20 mm apart on the same plate. Inoculum for fungi will be a 3 mm plug of agar from the advancing edge of a 4 to 7 day-old culture on 2.5% malt extract (ME) agar. Bacteria and yeasts will be inoculated with a 3 mm transfer loop in a streak 10 mm long. Antagonism studies will be done using 2.5% ME agar or other suitable media. In cases where bacteria do not grow on these media, the pH of the media will be adjusted to facilitate their growth.

After 5 to 7 days the distance *C. minor* grows toward the test organism will be measured. *C. minor* on a plate alone will be the control. Each test will be repeated 3 times. Data will be analyzed with a t-test. A positive test for antagonism will also include the presence of a zone of no growth between the 2 microorganisms.

[1/] Bridges, J. R. and J. E. Marler. 1978. A quantitative study of the microflora associated with the southern pine beetle. Study Plan FS-SO-2203-1.42.

Organisms that inhibit *C. minor* on agar plates will also be studied in the host tree. Spore suspensions of *C. minor* and the test organisms will be used to inoculate 40 cm loblolly pine billets in the laboratory. A No. 4 cork borer will be used to cut 4-6 inoculation holes in each billet. After inoculation the holes will be sealed with paraffin. The inoculation points will be equidistant from either end of the billet and equally spaced around the billet. Half of the inoculation points will receive *C. minor* plus the test organism. The other points will receive *C. minor* alone as controls. Inoculum for *C. minor* and each test organism will contain an equal concentration of spores. After three weeks the outer bark will be removed from the inoculated billets, and the distance *C. minor* grows in the inner bark will be measured. Each bolt will be a replicate; there will be 5 replicates. A t-test will be used to analyze the data. *C. minor* will also be observed for changes in form, appearance, and differences in amount of sporulation compared to controls.

To prepare the spore suspension inoculum, 2 ml of sterile 0.85% NaCl will be pipetted onto the surface of an agar plate of fungus, yeast, or bacterium and shaken to suspend the spores or cells. The spore concentration will be determined with a Newbauer counting chamber. The spore suspension will be diluted with saline to produce a suspension with approximately 10^6 spores/ml.

Those organisms that inhibit *C. minor* in bolts will be tested in the presence of the SPB. Emerging beetles will be surface sterilized and fed an antibiotic diet to remove associated microorganisms except the mycangial fungi (Marler and Barras 1978). The 3 organisms (*C. minor*, antagonist, SPB) will be inoculated together in a bolt. Microorganisms will be inoculated as a spore suspension as described above.

Measurements to be taken include: length of egg gallery, number of egg niches, and number of progeny produced. Each gallery will be considered a replicate. The control will be untreated emerging beetles inoculated in a bolt alone. A t-test will be used to compare the 2 groups with at least 15 galleries (replications) of each.

PRESENTATION OF RESULTS

A final report and manuscript will be prepared upon completion of the study.

PERSONNEL ASSIGNMENT

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